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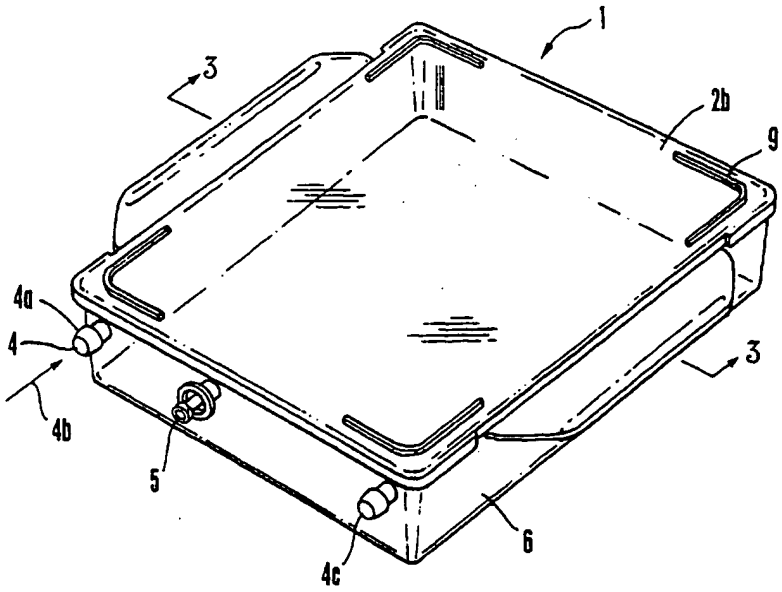
(54) Title: CHAMBER FOR LASER-BASED PROCESSING



(57) Abstract: A chamber (1) for presenting particles (15) having a bottom interior surface (2a) that is substantially planar, where at least 99.5 % of the surface is free from optical distortion. For example, when the chamber has side walls (6), they can be molded with the bottom portion (2) as a single piece. Consequently, at least 99.5 % of the particles are accessible to externally originating electromagnetic radiation. Also provided are methods for using the chamber to present biological specimens for imaging and to permit laser energy to be directed at selected particles, such as malignant cells.

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CHAMBER FOR LASER-BASED PROCESSING

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to transparent containers and more particularly to a chamber for laser-based processing.

BACKGROUND INFORMATION

A growing number of medical therapies involve removing a sample of the patient's cells prior to treatment, and then returning the cells to the patient after treatment. For example, sensitive stem cells can be removed and stored before chemotherapy, which might otherwise kill the cells. After chemotherapy, the cells are reintroduced to the original patient, serving as a stem cell transplant. Using the patient's own cells avoids the problems of rejection associated with transplants from a different person.

However, the sample of cells removed prior to treatment may harbor a number of malignant cells, so that returning the sample can inadvertently reintroduce the malignant cells. As a result, the malignant cells may reproliferate in the patient's body, causing the cancer to recur. Indeed, studies have shown that the more malignant cells remain in the sample, the greater the chance of recurrence. Thus, various techniques have been tried to remove or neutralize the malignant cells within the sample before it is returned to the patient.

In one technique, the sample is selectively stained to identify the malignant cells. These cells are then transferred to a chamber that is transparent so the stained cells can be imaged from outside by a camera. Once the malignant cells are identified, a laser is then directed through the chamber walls to kill the stained malignant cells. After the malignant cells have been eradicated by the laser, the sample is returned to the patient.

Unfortunately, previously available containers have blind spots and other optical imperfections, allowing a significant number of malignant cells to evade detection. Consequently, these containers can allow significant numbers of malignant cells to go undetected and be reintroduced into the patient. Thus, there is a need for a container, having exceptional optical qualities, that permits virtually all of the malignant cells in the sample to be accessible. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a chamber for laser-based processing. The chamber has a bottom interior surface that is substantially planar, where at least 99.5% of the surface is free from optical distortion. Consequently, when particles are introduced into the chamber, at least 99.5% of the particles are optically accessible from external points of view. For example, when the chamber has side walls, they can be molded with the bottom portion as a single piece. This allows the particles to be subjected to a

variety of applications, including fluorescence imaging and laser-induced necrosis.

The present invention also provides methods for presenting biological specimens in the chamber. For example, a specimen from a patient that contains malignant cells can be introduced into the chamber. Malignant cells imaged and identified by selective fluorescent labeling can then be killed by laser energy passing through the walls of the chamber. By virtue of the chamber's optical properties, virtually all of the malignant cells in the specimen can be eradicated, so that the specimen can be reintroduced into the patient virtually free of malignant cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a three-quarter view of one embodiment of a chamber of the invention.

Figure 2 shows a top view of a chamber.

Figure 3 shows a side view of a chamber according to the cross-section arrows 3 indicated in Figure 1.

Figure 4 shows a close-up view of the corner between a bottom portion and a side wall.

Figure 5 shows a close-up view of a first chamber stacked on a second chamber.

The following identification numbers are used in the Figures and throughout the specification:

1: chamber of the invention

2: bottom portion
2a: bottom interior surface
2b: top portion

3: indicates location and direction of cross-sectional view in Figure 3

4: port
4a: septum
4b: axial direction of the port
4c: second port

5: vent

6: side wall
6a: corner for measuring corner radius between bottom interior surface and a side wall
6b: corner for measuring corner radius between two side walls
6c: angle between side wall and bottom interior surface

7: a transmissible portion (for illumination)
7a: another transmissible portion (for laser energy)

8: handle
8a: tactile ridge

9: stacking alignment guide

10: stacking protrusion

11: spatial indexing marker

12: labeling area

13: scratch-resistant layer

14: external point of view

14a: line of sight of external point of view

15: particles

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a chamber for laser-based processing **1**. As shown in **Figures 1** and **3**, the chamber is closed and comprises a port **4** and a bottom portion **2** having a substantially planar interior surface **2a**. The term "comprising" as used herein, including its use in the body of the claims, is intended to be open-ended, thereby encompassing the recited elements or steps, as well as encompassing embodiments having additional elements or steps. By virtue of the design of the chamber, at least 99.5% of the bottom interior surface is optically accessible to an external point of view.

Certain features of the chamber are more easily disclosed and illustrated by way of an example using a particular method of using the chamber. This method is also part of the present invention. However, the method of using the chamber should not be considered a limitation on the device itself.

As an example, the chamber can be used as a container for killing an undesirable subgroup of

biological specimens within a larger specimen, such as a subgroup of malignant cells present in a specimen of stem cells. A suspension of stem cells or other biological specimen is first introduced into the chamber through a port **4**. To prevent the closed chamber from bulging from the introduced volume, a substantially equal volume of gas can be withdrawn from the chamber. Excess gas can also escape through an optional vent **5**.

The cells are then allowed to settle on the interior surface **2a** of the bottom portion **2**. Optionally, this settling step can be facilitated by centrifugation. For this reason, the chamber can be reinforced to withstand up to 1000 x *g* with structural ribbing or other conventional means. Aside from its structural features, however, the chamber's design and optical properties provide that virtually all of the cells will then be optically accessible to various external devices.

One such external device is a camera. Because the suspension has settled into a thin layer on the bottom interior surface, the particles have a relatively shallow focal depth, which is ideal for viewing by a camera outside the chamber. An external light source can also be directed to the cells so that they are fully illuminated for the camera.

If the cells have been treated with fluorescent markers to identify malignant cells, a light source of a predetermined wavelength can be used to cause the malignant cells to fluoresce. As a result, the camera can identify the malignant cells from the other cells. Once undesirable cells have been

identified, they can be killed by directing focused electromagnetic energy, such as from a laser, through the walls of the chamber to the cells.

Finally, the cell sample can be withdrawn from the chamber. To avoid cross-contamination, the sample is preferably withdrawn through a different port than the one used to introduce the specimen. The specimen, having its undesirable cells eradicated, can then be reintroduced into the patient. Certain nonessential aspects of this method are described in greater detail in pending applications 09/049,677, 09/451,659, 09/524,164, and U.S. Patent No. 5,874,266, each of which are incorporated herein by reference, including the references cited therein.

As disclosed above, a specific application for the chamber is for use with a laser to kill certain cells. With ultraviolet lasers, the directed energy can kill cells by thermal heating. The cells can also be treated with a dye to absorb the particular wavelengths of laser light more efficiently. For example, FD&C red #40 (allura red) can be used to absorb light at 523 nm. Thus, use of an energy-absorbing dye can reduce the amount of energy needed to kill a targeted cell.

This and other applications can be useful as a part of cellular therapies involving hematopoietic stem cells such as bone marrow or mobilized peripheral blood transplantation, immunotherapy for cancer and infection diseases, chondrocyte therapy for cartilage defects, neuronal cell therapy for neurodegenerative diseases and various other stem cell therapies.

Morever, use of the chamber is not limited to applications that kill cells. Other applications include using laser light for photomechanical disruption, photodissociation, photoablation and inducing photochemical reactions. A photosensitive substance within the cell mixture can also be used to treat specific cells by irradiation. Laser light can be used to make small transient pores in cell membranes, allowing the entry of genetic or other materials. Furthermore, specific molecules in or on the cell, such as proteins or genetic materials can be inactivated by laser light.

The chamber can also be used for nonclinical applications. For example, the chamber can be used to identify and precisely quantitate cells of interest. The chamber can further be used to purify selected cells, such as cells containing particular nucleic sequences of interest for genomic profiling or cells having expression profiles of interest for use in proteomics.

The chamber can also be used in nonbiological uses, for example identifying and sorting radioactive particles, where thoroughness of an application is considered important. Thus, the chamber need not be limited to targeting selected wanted or unwanted particles, but can be used in any application for inducing a response in selected particles from a larger sample, where it is desirable to affect virtually every such selected particle.

To perform in these applications, the chamber must first serve as a closed chamber for the particles. The term "chamber" as used herein means any solid configuration for containing a sample within its

interior. A useful volume range for the chamber interior is between 1 ml and 5000 ml, particularly between 10 ml and 2000 ml, and more particularly between 100 ml and 1000 ml. As used throughout herein, the term "between" is intended to be inclusive, meaning between and including both ends of a range.

The chamber is considered "closed" when it does not permit unintentional entry of outside substances, such as air-borne particulates, into the interior of the container. At the same time, the closed chamber contains the sample securely in its interior and does not allow the sample to leak when held in varying orientations.

The sample contained within the chamber will depend on the desired application and can be any solid or fluid. For many applications, however, the sample will be solid or semisolid particles in a fluid, such as a liquid or gas. For example, the particles can be biological or nonbiological in origin and can be suspended in the fluid. The term "biological" when applied to specimens includes liposomes, organelles, single and multiple cells, multicellular structures, tissues, embryos and organisms. The term further includes nucleic acids, proteins, lipids and their combinations. The source of these specimens can be viral, prokaryotic, eukaryotic, from plants, mammalian, human, or any combination of these sources. For example, it can be useful to use the chamber to target human cells detectably infected with bacteria or viruses.

To introduce the sample into the chamber, it has at least one port **4**. After a particular application, however, it may be desirable to withdraw

the sample through a separate second port **4c**, for example to avoid any cross-contamination between traces of treated and untreated specimens that may still harbor malignant cells.

A port can be an elongated hollow protrusion from a wall of the chamber, or can be as simple as an opening in a wall of the chamber, as long as it permits the passage of the sample. For example, the sample can be passed by pipetting, pouring or decanting through the port. Moreover, the port can preserve the integrity of the container by having a means for sealing or reversibly resealing the port, such as a lid, cover, pierceable seal or a screw cap threaded to the port.

A particularly useful means for sealing the port is a septum **4a**, such as an elastomeric plug. The septum can generally provide a leak-resistant seal, but can also be pierced with a hypodermic-type needle, cannula or other tube to pass samples through the septum. After the sample has been transferred, the cannula can then be withdrawn from the septum, restoring the original leak-resistant seal. A particularly useful septum is an aseptic or sterilizable septum, such as one that can be cleaned with alcohol or iodine swabs. This can allow patient samples to be introduced or withdrawn in open room air without requiring a laminar air-flow hood.

A port can be positioned anywhere on the walls of the chamber as long as it does not interfere with the chamber's optical qualities, discussed below. For example, the port can be at the top or on the sides of the chamber, and positioned at any angle. However, it can be particularly useful for the port to be

positioned at or adjacent to a corner of the chamber near the intersection of two or more intersecting surfaces. By tilting the chamber, the sample can then collect by funneling action near the port. This allows the sample to be conveniently withdrawn while minimizing the reach of the cannula into the interior of the chamber. The efficiency of the collection can be enhanced by providing a conical surface or other funneling feature near the port.

The port can also be configured to avoid or minimize scratching of the bottom interior surface when a cannula is inserted. If an elongated port is described as having an axial direction **4b** along which the cannula is inserted, the port can be positioned in the chamber so that the axial orientation of the port is relatively parallel to the bottom interior surface. The port can also be elongated further along the axial orientation to restrict the mobility of the cannula when inserted. As a result, a cannula inserted through the port is less likely to scratch or pit the bottom interior surface, interfering with its optical accessibility.

Introducing a volume of sample through an airtight port into a closed chamber can cause pressure to accumulate in the interior, possibly causing the chamber to bulge outward. Consequently, it can be useful for the chamber to also have a vent **5** to allow the release of excess gas and substantially equalizing the internal pressure to ambient pressure. A variety of gas-permeable vents are commercially available and can be filtered to prevent the inward passage of extraneous particulates and biological components, such as microorganisms including spores, bacteria and viruses. At the same time, the vent can prevent

outward leakage of sample within the container. Moreover, by allowing the gas to escape, the geometry of the chamber is more easily maintained, including the planarity of the bottom interior surface.

The bottom interior surface is where the particles settle substantially uniformly after being introduced. While the chamber can be held in any orientation when introducing the sample, the bottom portion should be considered the relatively horizontal portion that is closest to the ground when used. Finally, its design should allow the particles to settle on the bottom interior surface within a relatively shallow focal depth.

Useful areas for the bottom interior surface for most applications can be between 13 cm^2 and 5000 cm^2 in area. Other useful area ranges are between 0.01 cm^2 and 5000 cm^2, between 1 cm^2 and 2000 cm^2, and between 50 cm^2 and 1000 cm^2. These area ranges can be useful for treating specimen sizes up to 10 billion cells. When using the chamber with a very large number of cells, it becomes especially important that the bottom interior surface be as flat as possible to avoid welling the cells unevenly in the center of the bottom so that they overlap excessively.

The term "substantially planar" as used herein can be described in terms of an aspect ratio of planarity. First, the longest linear dimension of the bottom interior surface is measured. For example, in rectangularly shaped chambers, this generally means the length of the diagonal across the rectangular bottom interior surface. For a cylindrical chamber, the longest linear dimension is the diameter of the circular bottom interior surface. Second, the maximum

deviation of the bottom interior surface from perfect planarity is measured in a direction orthogonal to the perfectly planar bottom interior surface. If necessary, any three points from the perimeter of the bottom interior surface can be used to establish the reference plane. The aspect ratio of planarity is then obtained when the maximum orthogonal deviation is divided by the longest linear dimension of the bottom interior surface. Useful ranges for this ratio are less than 0.003, less than 0.0017 and less than 0.001. Thus, while the bottom interior surface need not be perfectly planar, it should be substantially planar to prevent particles from settling unevenly and excessively overlapping.

The desirability of a substantially planar bottom interior surface extends to the periphery of the surface as well. Where the chamber has side walls **6** that abut the bottom interior surface, their connection should not detract from the planarity of the bottom interior surface. Many containers, such as standard tissue culture flasks, have bottom interior surfaces that curve up to meet the side wall. This curvature results in a significant blind spot of distortion that can run along the entire perimeter of the bottom interior surface.

The curvature between the bottom interior surface and a side wall can be measured in terms of a "corner radius" measured as the radius at the intersection of the bottom interior surface with any other surface **6a**. When considering the entire perimeter of the bottom interior surface, every point of connection with another surface should have a corner radius less than 0.02 inch. Other useful values are less than 0.01 inch, less than 0.005 inch and less than

0.003 inch. By comparison, the corner radius of the angle between adjacent side walls **6b** can be comparatively large, for example greater than 0.02 inch, 0.04 inch or 0.08 inch, which facilitates molding.

One way to achieve a low corner radius between the bottom interior surface and a side wall is to manufacture the bottom portion and side walls as a single unit, rather than molding them separately and joining them. The term "a single unit" as used herein means not assembled from separate parts and mechanically joined such as by ultrasonic welding, adhesion or gaskets, but rather manufactured from an integral piece.

To facilitate molding as a single unit, the internal angle **6c** between the side wall and the bottom interior surface can be 91° or greater--in other words having a draft angle more than 1° with respect to perfect perpendicularity. This allows the bottom portion and side walls to be released from the mold during manufacture, while discouraging particles from accumulating along the perimeter during use. Other useful interior angles are 90.5°, 92°, and 93°. Molding the bottom portion and side walls as a single unit is most likely to optimize its optical qualities, as well as produce a chamber substantially free from manufacturing artifacts that could interfere with the optical accessibility of the bottom interior surface.

Some of the optical qualities of the chamber are illustrated in **Figure 4**. As shown, particles **15** settle substantially evenly on the bottom interior surface **2a**. This allows the particles to be

"presented" as used herein by providing optical access to the bottom interior surface and the particles from a point outside the chamber. As a result of the substantially planar bottom interior surface and the low corner radius at the perimeter, more than 99.5% of the bottom interior surface is optically accessible to an external point of view. This surface can also be at least 99.7%, 99.9%, 99.95% or 99.99% optically accessible, depending on the particular design and manufacturing standards of the chamber.

The term "optically accessible" as used herein means that a point on the bottom interior surface or particles settled on that point are not blocked or hidden from a line of sight from an external point of view. Moreover, the line of sight should be substantially free of distortion such as reflection, diffraction, diffusion, absorption and fluorescence. In short, the bottom interior surface must be virtually free from blind spots that would block the view from an external point of view.

The term "external point of view" **14** as used herein means a predetermined point outside of the chamber having an unblocked sightline **14a** to the bottom interior surface. The sightline can approach the bottom interior surface directly from above or from below, through the bottom portion. The sightline can also be indirect, for example, reflected by one or more mirrors outside the chamber. In practice, there can be one, two, three, four or more external points of view representing any number of optical devices, for example an image capture system such as a camera, or a light source such as a laser. Moreover, several devices can be accessible via a single point of view, for example a lens shared by a laser and a camera through which light

can be emitted and received. By defining the optical accessibility of the bottom interior surface in terms of a generic external point of view, the chamber is therefore not limited to any particular application.

Because the external point of view is outside the chamber and the bottom interior surface is inside the chamber, the sightlines necessarily pass through a portion of the chamber. Therefore, it can be useful to define one or more transmissible portions in the chamber. The term "transmissible portion" **7** as used herein describes a portion of the chamber that stands between the bottom interior surface and the external point of view.

The transmissible portion can stand between only a portion of the bottom interior surface and the external point of view, or the entire bottom interior surface. When the external point of view is below the chamber, the transmissible portion can be part of the bottom portion **2**. Similarly, the transmissible portion can be at the top of the chamber **2b** when the external point of view is above the chamber.

When the external point of view is used to illuminate the bottom interior surface, a useful transmissible portion can be substantially transparent to at least one wavelength of electromagnetic radiation between 100 nm and 1000 nm. Other useful wavelength ranges include between 200 nm and 800 nm, between 320 nm and 695 nm, and between 330 nm and 605 nm. In **Figure 3**, the transmissible portion **7** for purposes of illuminating the bottom interior surface can be on either the top portion **2b** or the bottom portion **2**. As shown, however, when the desired application is

illumination over a large area without a specific line of sight, minor features can be incorporated such as the stacking alignment guide, as long as they do not interfere with the application. For example, a line of sight can be selected **14a** so that it is not blocked by the stacking alignment guides **9**. In applications where the depth of focus is relatively shallow, such as imaging, minor features far outside the focal plane, such as the stacking alignment guides, can also be substantially noninterfering.

A transmissible portion can also be defined when a laser is used to direct energy to the bottom interior surface. In **Figure 3**, for example, the transmissible portion **7a** is the bottom portion **2**. When the external point of view comprises a laser for directing energy to the bottom interior surface, a useful transmissible portion can be substantially transparent to at least one wavelength of electromagnetic radiation between 100 nm and 30 micrometers. This range covers various laser wavelengths such as 349 nm, 355 nm, 488 nm, 523 nm, 532 nm, 580 nm, 590 nm, 633 nm, 1064 nm, 2100 nm and 2940 nm. Other useful ranges are between 150 nm and 3.5 micrometers, and between 330 nm and 2.5 micrometers. However, the transmissible portion and the containment qualities of the chamber itself should not be affected by any heat generated by the laser for the particular application.

Useful materials for making the transmissible portions of the chamber include polycarbonate, polyolefin, polysulfone and cycloolefin. Useful cycloolefins are described in U.S. Patents No. 5,910,287 and No. 6,063,338, both of which are

incorporated herein by reference, including the references cited therein. Such cycloolefins are examples of materials where the material has relatively low intrinsic fluorescence, which might otherwise interfere with fluorescence applications on samples within the chamber. Thus, the fluorescence of the material can be less than 50%, 25% or even less of the fluorescence of a reference material of fused silica of comparable thickness, where the relative fluorescence is measured by standard methods, including those described in these two patents.

A particularly useful material for making the transmissible portions of the chamber is medical grade general purpose polystyrene (GPPS), such as those found in the GPPS product lines STYRON (Dow Chemical Co.; Midland, MI), POLYSTYROL (BASF; Ludwigshafen, Germany), REPLAY (Huntsman Corp., Salt Lake City, UT) or DYLENE (Arco; Los Angeles, CA). Various types of polystyrenes are available commercially, but the particular material that is used in the chamber will depend, of course, on the particular application for the chamber.

For example, when using the chamber with specific wavelengths of light, the material should be selected to be substantially nonabsorbing at that wavelength. Similarly, when using the chamber with fluorescence-based applications, the material should be selected to be substantially non-autofluorescent so as not to affect or interfere with the detection of fluorescence from the sample itself.

The transmissible portion can be less than 0.5 inch thick, less than 0.2 inch thick, or less than 0.1 inch thick. The transmissible portion and the rest

of the chamber should also be constructed solidly enough to withstand normal laboratory conditions and handling.

The material of the bottom interior surface can also be selected or treated so that it is biocompatible and noncytotoxic. The surface, as well as the entire interior of the chamber can also be sterile to avoid introducing any contamination into the sample. Thus, the transmissible portion can be selected to be a material that is sterilizable, for example by radiation or ethylene oxide, without substantially affecting the optical accessibility of the bottom interior surface.

The surface of the bottom portion can also be treated to increase wettability. This is a common procedure with many tissue culture plastics and can be achieved by standard plasma treatments. This has the advantage of decreasing the water contact angle and promoting a uniformly thin layer of sample on the bottom interior surface. A portion of the interior surface of the chamber other than the bottom interior surface can also be treated to increase wettability, or conversely to decrease wettability to discourage liquid samples from adhering to other portions.

Once the bottom and side walls have been molded as a single unit, they can be joined to the top portion **2b** by any standard means, including adhesion and ultrasonic welding. If desired, the entire chamber can then be sterilized as a whole.

The chamber can have several other features to facilitate its use. Each of these features can be implemented in a variety of ways and positions on the

chamber, as long as they do not interfere with the optical accessibility of the bottom interior surface.

For example, the chamber can have one or more handles **8** to allow users to hold and manipulate the chamber without touching the bottom portion. The handle can also have tactile ridges **8a** to promote secure gripping of the chamber. Handles provide an added advantage when molding the chamber because the handles need not have the same optical qualities as the other portions of the chamber. For example, when molding the bottom portion and side walls, the part of the mold corresponding to one handle can be used as the point of injection for the resin and the part of the mold corresponding to another handle can have a vent to allow the air to escape. As a result, any optical imperfections that should be incurred during the manufacturing process can be relegated to the handles, rather than the bottom portion. Similarly, ejection pins, if necessary, can be positioned at the handle or along the perimeter of the stacking protrusion, so that they do not interfere with the optical accessibility of the bottom interior surface.

To further protect the bottom portion, during manufacture, shipping and handling, the chamber can have a scratch-resistant layer **13** removably adhered to the exterior of a transmissible portion. For example, the layer can be a peel-away plastic film or a separate rigid cover

For practical reasons, it is also desirable to allow chambers to be stacked without affecting the optical qualities of each chamber. Thus, the upper exterior surface of a chamber can have a stacking

alignment guide **9**. Correspondingly, the lower exterior surface of the chamber can have a stacking protrusion **10**. The stacking protrusion preferably extends farther than the stacking alignment guide so that the stacking alignment guide does not scratch the bottom portion.

As seen in **Figure 5**, when a first device is stacked on a second device, the stacking protrusion of the first device can contact the stacking alignment guide of the second device, either directly or indirectly. In this embodiment, the stacking alignment guides **9** are positioned inside relative to the stacking protrusions **10**. However, the stacking alignment guides can also be positioned outside relative to the stacking protrusions. These or similar configurations allow the chambers to be stacked vertically, resisting horizontal motion such as sliding or shifting.

The stacking protrusion **10** can further serve as a stand-off protrusion that extends lower than the bottom portion, allowing the chamber to be set on a horizontal surface while preventing scratches and scrapes to the bottom exterior surface. The stand-off protrusion can run along the perimeter of the lower exterior surface, or merely a portion of the perimeter. However, each of these stacking and stand-off features should be carefully designed to avoid interfering with the optical accessibility of the bottom interior surface.

When the chamber is used with an apparatus, it can also be useful for the chamber to have spatial indexing markers **11**, which can be any identifiable feature on the chamber to allow the apparatus to detect

whether the chamber is correctly positioned relative to the apparatus.

The chamber can also feature a labeling area **12**, for example a markable surface or identification marker. These markers can include alphanumeric identifiers, bar codes and other scannable labels, holograms and laser etchings. In **Figure 3**, the labeling area is shown on the exterior surface of one of the side walls.

A labeling area is particularly useful when a separate apparatus can obtain information about the chamber from the labeling area. For example, if various chambers can be used with the apparatus, the labeling area can contain apparatus-readable information specifying the particular type of chamber being used. The labeling area can therefore provide information about the properties of the chamber such as capacity, planarity, number of subchambers, wall thickness, optimal transmissible wavelengths, autofluorescence properties, identification of the sample and sample properties and other parameters of use to the particular application. In this way, use of the chamber can involve fewer steps and reduce operator errors.

When using the chamber with multiple samples, it may be useful for the chamber to be able to process more than one sample at a time. The chamber can therefore be divided into more than one subchamber, each subchamber containing a substantially planar bottom interior surface. In turn, each subchamber can have at least one port.

Although the invention has been illustrated by the embodiments discussed above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

We claim:

1. A device for presenting particles in a fluid, comprising

a closed chamber having at least one port;

a bottom having a substantially planar interior surface, wherein at least 99.5% of the bottom interior surface is optically accessible from at least one external point of view; and

at least one side wall, wherein the bottom and the side wall are a single unit.

2. A device for presenting particles in a fluid, comprising

a closed chamber having at least one port;

a bottom having a substantially planar interior surface, wherein at least 99.5% of the bottom interior surface is optically accessible from at least one external point of view; and

at least one side wall; wherein the area of the bottom interior surface is between 13 cm^2 and 5000 cm^2.

3. A device for presenting particles in a fluid, comprising

a closed chamber capable of containing particles, wherein the chamber has

at least one port;

a bottom having a substantially planar interior surface;

at least one side wall, wherein when particles contained in the chamber settle substantially uniformly on the bottom interior surface, at least 99.5% of the particles are optically accessible from at least one external point of view.

4. The device of claim 1, wherein the volume of the chamber is between 1 ml and 5000 ml.

5. The device of claim 1, wherein the volume is between 10 ml and 2000 ml.

6. The device of claim 1, wherein the volume is between 100 ml and 1000 ml.

7. The device of claim 1, further comprising a second port.

8. The device of claim 1, wherein the port further comprises a means for sealing the port.

9. The device of claim 1, wherein the port is pierceably sealed.

10. The device of claim **1**, wherein the port includes a septum.

11. The device of claim **10**, wherein the septum is aseptic.

12. The device of claim **1**, wherein the port is positioned adjacent to a corner or funneling feature of the chamber.

13. The device of claim **1**, wherein the axial orientation of the port is relatively parallel to the bottom interior surface.

14. The device of claim **1**, further comprising a vent.

15. The device of claim **14**, wherein the vent permits substantial equalization of internal pressure to ambient pressure.

16. The device of claim **14**, wherein the vent is filtered to prevent the passage of biological components and extraneous particulates.

17. The device of claim **16**, wherein the biological component is a cell.

18. The device of claim **16**, wherein the biological component is a microorganism.

19. The device of claim **1**, wherein the particles in the fluid settle substantially uniformly on the bottom interior surface when introduced into the interior.

20. The device of claim **1**, wherein the bottom interior surface is between 0.01 cm^2 and 5000 cm^2.

21. The device of claim **1**, wherein the bottom interior surface is between 1 cm^2 and 2000 cm^2.

22. The device of claim **1**, wherein the bottom interior surface is between 50 cm^2 and 1000 cm^2.

23. The device of claim **1**, wherein an aspect ratio of planarity is defined as the maximum orthogonal deviation from planarity of the bottom interior surface divided by the longest linear dimension of the bottom interior surface, and wherein the aspect ratio of planarity is less than 0.003.

24. The device of claim **23**, wherein the aspect ratio of planarity is less than 0.0017.

25. The device of claim **23**, wherein the aspect ratio of planarity is less than 0.001.

26. The device of claim **1**, wherein the perimeter of the bottom interior surface has a corner radius less than 0.02 inch.

27. The device of claim **26**, wherein the corner radius is less than 0.01 inch.

28. The device of claim **26**, wherein the corner radius is less than 0.005 inch.

29. The device of claim **26**, wherein the corner radius is less than 0.003 inch.

30. The device of claim 1, wherein the chamber has a top portion, a bottom portion having the bottom interior surface, and at least one side wall, wherein the bottom portion and side wall are molded as a single piece.

31. The device of claim 1, wherein the bottom interior surface is substantially free from manufacturing artifacts that interfere with the optical accessibility of the bottom interior surface.

32. The device of claim 1, wherein at least 99.7% of the bottom interior surface is optically accessible.

33. The device of claim 1, wherein at least 99.9% of the bottom interior surface is optically accessible.

34. The device of claim 1, wherein at least 99.95% of the bottom interior surface is optically accessible.

35. The device of claim 1, wherein at least 99.99% of the bottom interior surface is optically accessible.

36. The device of claim 1, wherein a transmissible portion of the chamber is defined between the bottom interior surface and an external point of view, wherein the transmissible portion is substantially transparent to a wavelength of electromagnetic radiation between 100 nm and 1000 nm.

37. The device of claim **36**, wherein the wavelength is between 200 nm and 800 nm.

38. The device of claim **36**, wherein the wavelength is between 320 nm and 695 nm.

39. The device of claim **36**, wherein the wavelength is between 330 nm and 605 nm.

40. The device of claim **36**, wherein the transmissible portion is at the top of the chamber.

41. The device of claim **36**, wherein the transmissible portion is at the bottom of the chamber.

42. The device of claim **1**, wherein a transmissible portion of the chamber is defined between the bottom interior surface and the external point of view, wherein the transmissible portion is substantially transparent to a wavelength of electromagnetic radiation between 100 nm and 30 micrometers.

43. The device of claim **42**, wherein the wavelength is between 150 nm and 3.5 micrometers.

44. The device of claim **42**, wherein the wavelength is between 330 nm and 2.5 micrometers.

45. The device of claim **42**, wherein the transmissible portion is composed of a resin selected from the group consisting of polycarbonate, polyolefin, cycloolefin and polysulfone.

46. The device of claim **42**, wherein the transmissible portion is composed of polystyrene.

47. The device of claim **42**, wherein the transmissible portion can be radiation-sterilized.

48. The device of claim **42**, wherein the transmissible portion can be sterilized by ethylene oxide gas.

49. The device of claim **42**, wherein the transmissible portion is at the top of the chamber.

50. The device of claim **42**, wherein the transmissible portion is at the bottom of the chamber.

51. The device of claim **42**, wherein the transmissible portion is less than 0.5 inch thick.

52. The device of claim **42**, wherein the transmissible portion is less than 0.2 inch thick.

53. The device of claim **42**, wherein the transmissible portion is less than 0.1 inch thick.

54. The device of claim **1**, wherein the bottom interior surface is biocompatible.

55. The device of claim **1**, wherein the interior of the chamber is sterile.

56. The device of claim **1**, wherein the bottom interior surface is treated to increase wettability.

57. The device of claim **1**, wherein a portion of the interior surface of the chamber other than the bottom interior surface is treated to decrease wettability.

58. The device of claim **1**, further comprising a handle that does not interfere with the optical accessibility of the bottom interior surface.

59. The device of claim **42**, further comprising a scratch-resistant layer removably adhered to the exterior of a transmissible portion.

60. The device of claim **1**, wherein the upper exterior surface further comprises a stacking alignment guide and the lower exterior surface further comprises a stacking protrusion that extends farther than the stacking alignment guide, but does not interfere with the optical accessibility of the bottom interior surface; wherein when a first device is stacked vertically on a second device, the stacking protrusion of the first device resists horizontal motion relative to the stacking alignment guide of the second device.

61. The device of claim **1**, wherein the device further comprises a stand-off protrusion that extends lower than the bottom portion.

62. The device of claim **61**, wherein the stand-off protrusion runs along at least a portion of the perimeter of the lower exterior surface.

63. The device of claim **1**, further comprising a spatial indexing marker.

64. The device of claim **1**, further comprising a labeling area.

65. The device of claim **64**, wherein the labeling area comprises a markable surface.

66. The device of claim **64**, wherein the labeling area comprises an identification marker.

67. The device of claim **1**, wherein the chamber is divided into more than one subchamber, each subchamber containing a substantially planar bottom interior surface.

68. The device of claim **67**, wherein each subchamber has at least one port.

69. A method for presenting biological specimens in a fluid, comprising the steps of

(a) obtaining a device comprising a closed chamber having at least one port;

a bottom having a substantially planar interior surface, wherein at least 99.5% of the bottom interior surface is optically accessible from at least one external point of view; and

at least one side wall;

(b) introducing the specimens in a fluid through the port into the device; and

(c) allowing the specimens to settle on the bottom interior surface;

thereby presenting greater than 99.5% of the specimens to an external point of view.

70. The method of claim **69**, further comprising the step of

(d) withdrawing from the chamber a volume of gas substantially equal to the volume introduced in step (b).

71. The method of claim **69**, wherein step (c) is performed by centrifugation.

72. The method of claim **69**, wherein a selected subset of the specimens is fluorescently labeled, and further comprising the step of

(e) directing electromagnetic radiation to the bottom interior surface, thereby causing the subset to fluoresce.

73. The method of claim **72**, further comprising the step of

(f) directing electromagnetic radiation to substantially only the subset that fluoresced in step (e).

74. The method of claim **69**, further comprising the step of

(g) withdrawing from the device the specimens in the fluid through a port of the device.

75. The method of claim **74**, wherein the ports of steps (b) and (g) are different ports.

Fig. 1

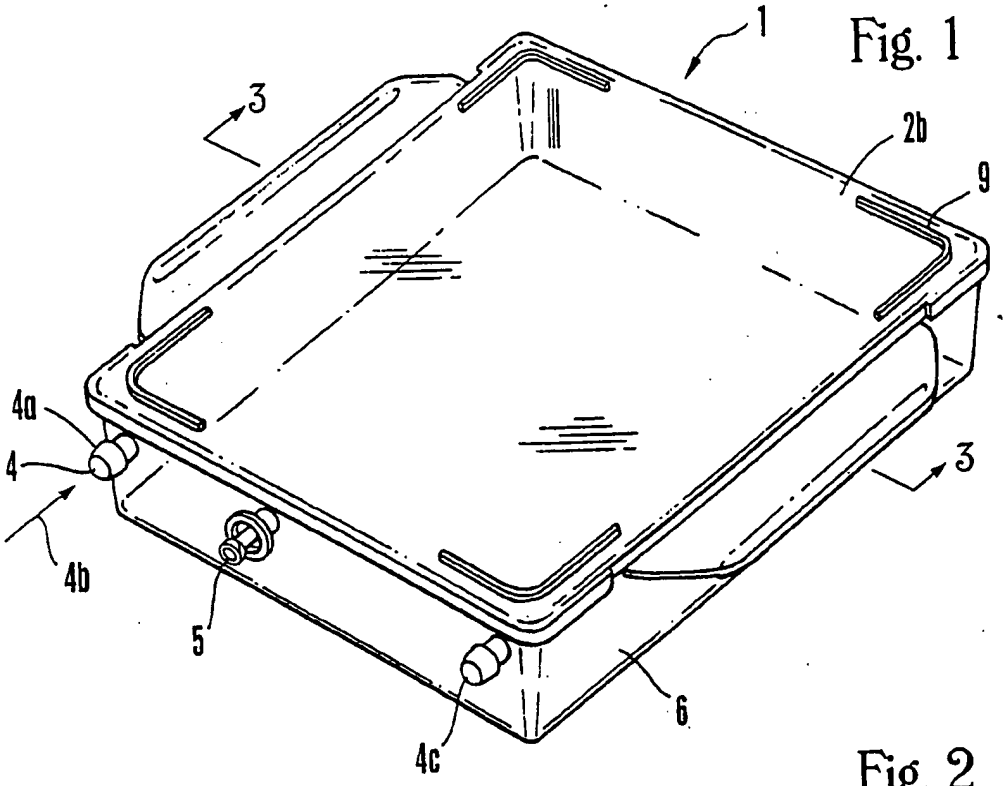
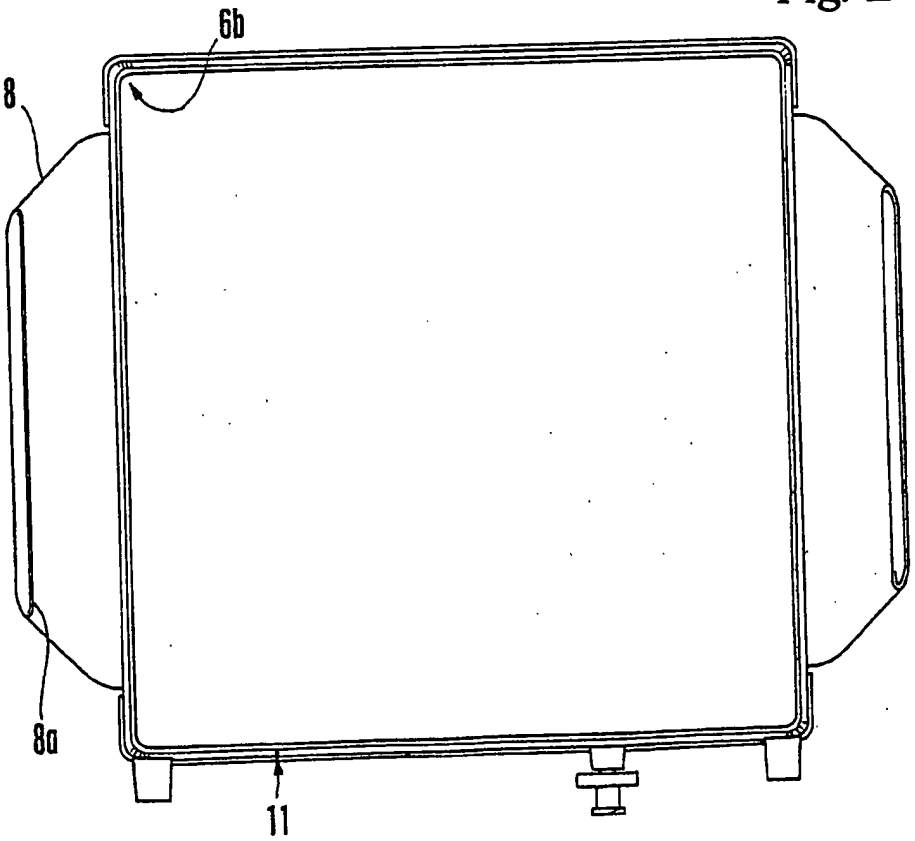


Fig. 2



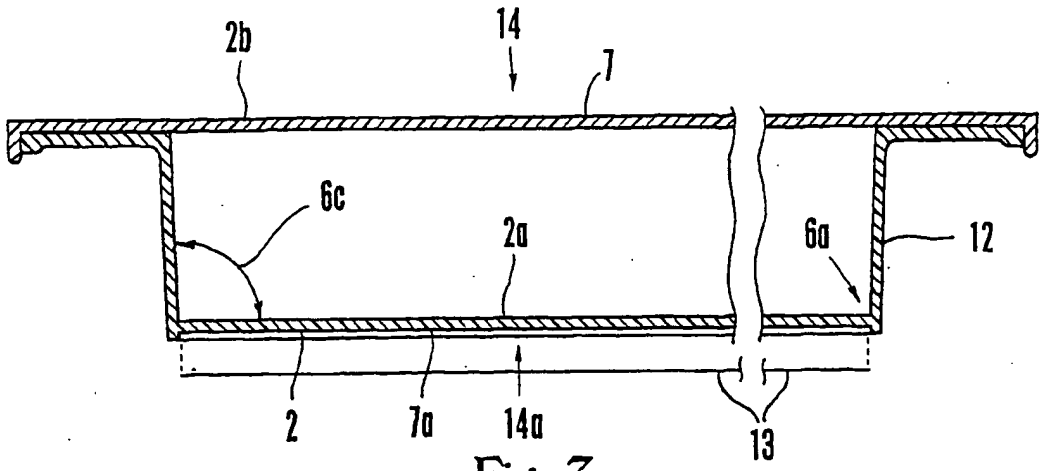


Fig. 3

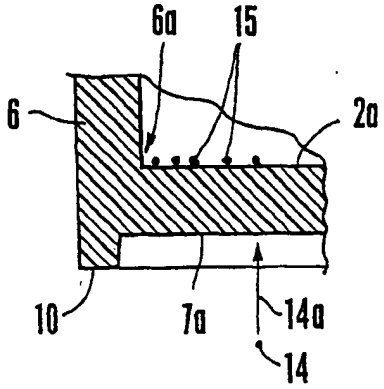


Fig. 4

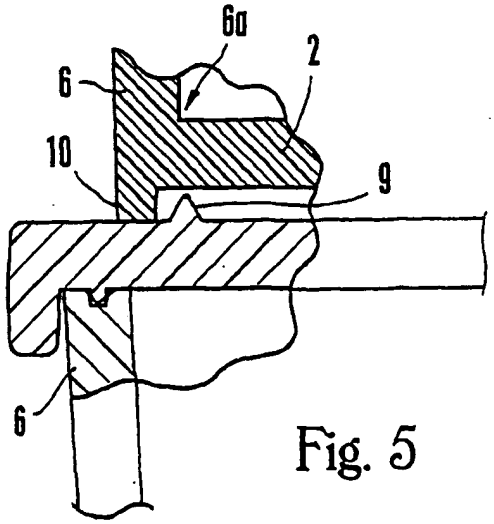


Fig. 5